Lasers in Surgery and Medicine 18:406–409 (1996)

Pharmacokinetics of Pyropheophorbide-a-Hexyl Ether in the Dog

John T. Payne, DVM, MS, Dudley L. McCaw, DVM, MS, Stan W. Casteel, DVM, PhD, Donita Frazier, DVM, PhD, Kevin Rogers, BS, MS, and Robert V. Tompson, PhD

Departments of Veterinary Medicine and Surgery (J.T.P., D.L.M.), Veterinary Pathology (S.W.C.), College of Veterinary Medicine, and the Department of Nuclear Engineering (K.R., R.V.T.), College of Engineering, University of Missouri-Columbia, Columbia, Missouri 65211; Department of Comparative Medicine (D.F.), College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee 37901.

Background and objectives. **Pyropheophorbide-a-hexyl ether (HPPH) is a new compound being investigated for use as a photosensitizer for photodynamic therapy; however, the pharmacokinetics are not known for any of the target species likely to be treated with this drug. The objective of this study was to determine the pharmacokinetic parameters of this drug prior to institution of a clinical trial in canine patients with various cancers.** ***Study design, Materials and Methods.*** **HPPH (0.3mg/kg I.V.) was administered to 12 dogs and blood samples were drawn at intervals for 24 hours and plasma HPPH concentrations were determined. Pharmacokinetic parameters were calculated for each dog.** ***Results.*** **No evidence of toxicity was noted in any dog. The mean half-life was calculated to be 26.98 ± 2.35 hrs. The mean clearance was 5.061 ± 0.214 ml/hr/kg. The mean volume of distribution of the central compartment was 0.069 ± 0.003 L/kg, and the mean steady state volume of distribution was 4.47 ± 0.25 L/kg.** ***Conclusion.*** **The conclusion is that 0.3 mg/kg HPPH injected intravenously resulted in measurable plasma levels for 24 hrs, and resulted in no detectable adverse reactions.**

Key words: pharmacokinetics, pheophorbide, photodynamic therapy, photosensitizer

INTRODUCTION

Photodynamic therapy (PDT) involves the use of light activated drugs that when injected into the body may be retained within tumor tissue in concentrations greater than or for longer periods of time than drug in adjacent normal tissue. The drug is activated by the appropriate wavelength of light, usually supplied by a laser. Following light activation, the drug causes the formation of singlet oxygen by energy transfer to endogenous oxygen, leading to necrosis of the tumor while sparing the normal tissue to varying degrees [1].

A number of photosensitizers, including Photofrin®, tin etiopurpurin, aluminum phthalocyanine, and various chlorins, have been used as photosensitizing agents. Photosensitizers for use in PDT require certain characteristics to be clinically useful. They should be nontoxic and rapidly eliminated from normal tissue (short half-life). The ideal drug should be activated at a wavelength within the range of 650–800 nm, because light in this wavelength range has a greater depth of penetration than shorter wavelengths in the visible spectrum, therefore allowing activa-

Accepted for publication March 10, 1995.

Address reprint requests to John T. Payne, DVM, University of Missouri-Columbia, College of Veterinary Medicine, Columbia, MO 65211.

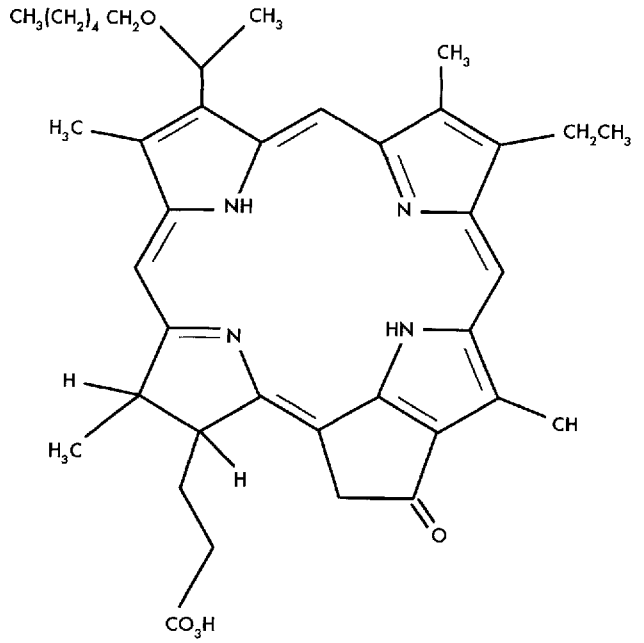

Fig. 1. Structure of HPPH.

tion of drug deeper within tumor. Following treatment, the drug should be eliminated from skin and subcutaneous tissue quickly since photosensitization to sunlight may be a problem following treatment. Additionally, the drug should be a single pure compound and produce high yields of singlet oxygen upon activation [2].

Pyropheophorbide-a-hexyl ether (2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a, HPPH) is a new photosensitizing agent proposed for used in PDT. HPPH is derived from pheophorbide a, obtained from *Spirulina* algae. Pheophorbide a is converted to the bromo compound, condensed with hexyl alcohol, pyrolized to form the pyro compound, and finally hydrolyzed to form the mono carboxylic acid [3]. Its structure is shown in Figure 1 [4].

While HPPH is not likely to be the ideal photosensitizing agent described earlier, it does have some of the desirable characteristics of the ideal drug. HPPH is a single pure compound, is nontoxic, and generates singlet oxygen at yields near 70% [3]. Furthermore, HPPH has been demonstrated to cause much less cutaneous photosensitivity than Photofrin [3]. While the antitumor efficacy of this compound has been clearly demonstrated in laboratory animals, further clinical and laboratory work needs to be done to determine the efficacy of HPPH in the treatment of naturally occurring tumors in animals and humans [3].

MATERIALS AND METHODS

Twelve young adult, mixed breed dogs were obtained and conditioned for 30 days according to the protocol of the Animal Care and Use Committee of the University of Missouri—Columbia. Six healthy male and six female dogs averaging 24.6 kg body weight were administered 0.3 mg/kg body weight HPPH intravenously via the cephalic vein. Jugular vein blood samples were obtained from each dog prior to and at 1, 2, 3, 4, 6, 8, 12, 16, and 24 hours after administration of the drug. Blood was collected into a 3 ml blood tube containing EDTA, immediately centrifuged, and the plasma was separated using disposable micropipettes. The plasma samples were immediately frozen and stored at −70°C. After all blood samples were collected, they were shipped by overnight mail on dry ice to the University of Tennessee for analysis of HPPH levels. Blood and plasma samples were protected from exposure to light to the best extent possible.

HPPH was extracted from plasma with acidic ethanol (pH 4.1). After shaking for 30 minutes and centrifugation, the supernatant was transferred to a clean tube and evaporated to dryness under nitrogen. HPPH was redissolved in 1 ml ethanol and fluorescence was read on a spectrofluorophotometer (Shimadzu model 5000, excitation 412, emission 500–1,000). The sensitivity of the assay was 0.05 μg/ml and the recovery of HPPH from plasma was 95%.

Plasma HPPH concentrations were fitted to a two-compartment model and pharmacokinetic parameters were determined using an automated curve-stripping program (RStrip, Micromath Scientific Software, Salt Lake City, UT). Pharmacokinetic parameters were calculated as follows: half-life ($t_{1/2}$) = ln 2/b, where b is the slope of the elimination phase. Volume of distribution of the central compartment (V_C) = dose/A + B, where A and B are the intercepts of the distribution and elimination phases, respectively. Volume of distribution at steady state (VSS) = dose $[(A/a)^2 + (B/b)^2]/[(A/a) + (B/b)]^2$. Clearance (Cl) = dose/AUC, where AUC (area under the elimination curve) was calculated by the trapezoidal method.

RESULTS

All dogs tolerated the drug well, and no signs of acute toxicity, such as vomiting, diarrhea, or salivation, were noted. Animals were fed after the 12 hour blood sample was taken and water was

available at all times; all animals ate and drank normally throughout the experiment. These observations support unpublished results of a previously performed acute toxicity study (TJ Dougherty, personal communication, 1994).

The HPPH plasma concentration vs. time profiles for all dogs with a predicted plasma HPPH concentration vs. time line generated from the mean rate constants (α and β) and intercepts (A and B) are shown in Figure 2. The curve fits well to a two compartment model, and pharmacokinetic values for each dog and mean values ± standard error of the mean (SEM) are noted in Table 1. Mean half-life ($t1_{/2}$) was determined to be 26.982 ± 8.161 hours. Mean clearance was 5.061 ± 0.744 ml/hr/kg. The mean value for volume of distribution (V_C) was 0.069 ± 0.012 l/kg, and the mean steady state volume of distribution (V_{SS}) was 4.468 ± 0.848 l/kg.

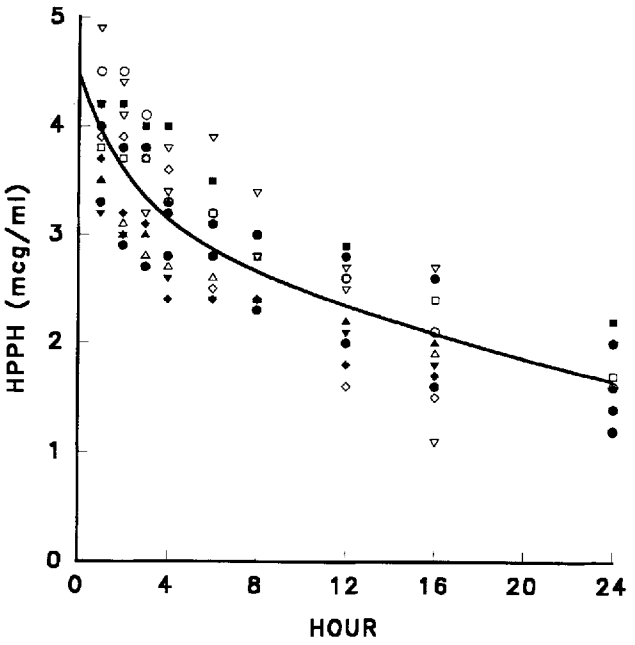

Fig. 2. HPPH plasma concentration vs. time profiles for all dogs with a predicted plasma concentration vs. time line generated from the mean rate constants (α and β) and intercepts (A and B).

DISCUSSION

Pyropheophorbide appears to be a safe drug, causing no noticeable side effects in any dogs in the present study. The animals were housed indoors, which may have precluded development of cutaneous photosensitivity to sunlight. Similarly, the acute toxicity studies performed in dogs by Dougherty and colleagues found no evidence of significant acute toxicity. The present experiment was of short duration, and chronic side effects of HPPH would not have been noted in this study.

The half-life of this drug in dogs was 26.982 ± 8.161 hours. This is slightly longer than the elimination half-life that was reported for HPPH in mice (21 hours) [3]. Comparatively, the Photofrin II clearance curve in mice best fit a triexponential equation with elimination half-lives of 0.75, 10, and 220 hours [5]. There are clinical reports describing use of both Photofrin II and chloroaluminum sulfonated phthalocyanine for PDT in dogs [6–8]; however, no pharmacokinetic data in dogs have been reported on any drug being evaluated or used as a photosensitizer.

The volume of distribution for this drug in the central compartment was 0.069 l/kg and the

TABLE 1. Individual Pharmacokinetic Values for Dogs With Calculated Means and Standard Error of the Mean

Dog no.	C_{max} (μg/ml)	t½ (hr)	AUC (μg/ml/hr)	A (μg/ml)	α(hr^{-1})	B (μg/ml)	β (hr^{-1})	CL (ml/hr/kg)	V_C (l/kg)	V_{SS} (l/kg)
1	4.44	31.06	68.23	3.56	0.022	0.87	0.437	4.397	0.068	4.43
2	4.77	35.69	64.76	3.11	0.019	1.66	0.331	4.633	0.063	4.47
3	3.72	23.73	50.95	2.88	0.029	0.84	0.688	5.888	0.081	3.72
4	4.04	22.18	64.23	3.74	0.031	0.29	0.265	4.671	0.074	4.03
5	4.81	29.09	72.69	3.84	0.024	0.97	0.378	4.127	0.062	4.81
6	4.66	21.41	52.83	3.08	0.032	1.58	1.105	5.679	0.064	4.66
7	3.53	39.96	55.81	2.28	0.017	1.26	0.104	5.375	0.085	3.54
8	4.28	13.36	53.92	4.07	0.061	0.20	0.052	5.564	0.070	4.27
9	4.29	39.18	51.61	2.35	0.018	1.94	0.346	5.813	0.070	4.29
10	5.16	19.81	65.24	3.74	0.035	1.42	0.317	4.598	0.058	5.16
11	3.28	23.53	49.59	2.84	0.036	0.55	0.135	6.049	0.089	3.39
12	6.55	24.78	76.25	4.24	0.028	2.31	1.034	3.934	0.046	6.55
Mean ± SEM	4.46 ± 0.25	26.98 ± 2.36	60.51 ± 2.65	3.31 ± 0.19	0.029 ± 0.003	1.16 ± 0.19	0.435 ± 0.0002	5.061 ± 0.214	0.069 ± 0.003	4.47 ± 0.25

steady-state volume of distribution is 4.47 l/kg, indicating a large volume of distribution beyond the central compartment. This large distribution of the drug is desirable to allow the drug to reach tumors in many different tissue types. No data relating to volumes of distribution of other photosensitizers could be found for the dog for comparative purposes.

Clearance of HPPH in the dog was found to be 5.061 ml/hr/kg. This demonstrates rather rapid clearance from plasma and rapid distribution to other tissues. In the mouse, HPPH sequesters in the highest concentrations in tissues with high reticuloendothelial cell populations and can be detected for as long as 7 days postadministration. In the mouse, drug elimination occurs through the feces. The route of elimination is not known in the dog.

Currently, clinical trials are underway using HPPH (0.3 mg/kg) to photosensitize dogs for photodynamic therapy. Based on these data, this appears to be a safe dose for delivering a measurable quantity of HPPH to dogs with tumors. This dosage resulted in no detectable adverse reactions and in measurable plasma concentrations for at least 24 hours.

ACKNOWLEDGMENTS

The authors thank Dr. Thomas J. Dougherty for providing the HPPH and for technical advice during the project. This study was supported by the following grants: NIH R R01192 and Office of Naval Research N00014-91-0134.

REFERENCES

1. Weishaupt KR, Gomer CJ, Dougherty TJ. Identification of singlet oxygen as the cytotoxic agent in photoinactivation of a murine tumor. Cancer Res 1976; 7:2326–2329.
2. Ash DV, Brown SB. New drugs and future developments in photodynamic therapy. Eur J Cancer 1993; 29A:1781–1783.
3. Bellnier DA, Henderson BW, Pandey RK, Potter WR, Dougherty TJ. Murine pharmacokinetics and antitumor efficacy of the photodynamic sensitizer 2-[hexyloxyethyl]-2-devinyl pyropheophorbide-a. J Photochem Photobiol B 1993; 20:55–61.
4. Sumlin AB, Dougherty TJ, Smith KM. Structure/activity relationship among photosensitizers related to pheophorbide and bacteriopheophorbide. Bioorg Med Chem Lett 1992; 2:491–496.
5. Bellnier DA, HO Y-K, Pandey RK, Missert JR, Dougherty TJ. Distribution and elimination of Photofrin II in mice. Photochem Photobiol 1989; 50:221–228.
6. Cheli R, Addis F, Mortellaro CM, Fonda D, Cubeddu R. Photodynamic therapy of spontaneous animal tumors using the active component of hematoporphyrin derivative (DHE) as photosensitizing drug: Clinical results. Cancer Lett 1987; 38:101–105.
7. Roberts WG, Klein MK, Loomis M, Weldy S, Berns MW. Photodynamic therapy of spontaneous cancers in felines, canines, and snakes with chloro-aluminum sulfonated phthalocyanine. J Natl Cancer Inst 1991; 83:18–23.
8. Dougherty TJ, Thoma RE, Boyle DG, Weishaupt KR. Interstitial photoradiation therapy for primary solid tumors in pet cats and dogs. Cancer Res 1981; 41:401–404.